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Phytochemical Screening And In - *Vitro* Antioxidant Activity Of *Caesalpinia Bonducella* L.,

KEYWORDS: *Caesalpinia bonducella*, Anti-oxidant activity, Phytoconstituents

P Rajalakshmi
PG & Research Department of Botany, Government arts college Coimbatore-18

V Vadivel
Department of CARISM, SASTRA University, Thanjavur.

G Subhashini
Department of Biotechnology, SNR & Sons College, Coimbatore

M Pugalenthi
PG & Research Department of Botany, Government arts college Coimbatore-18

ABSTRACT *Plants have been an important source of medicine for thousands of years. Even today, the world health organization estimates that up to 80% of people still rely mainly on traditional remedies such as herbs for their medicines. Medicinal plants constitute one of the main sources of new pharmaceutical and health care products. In this study seeds and leaves of Caesalpinia bonducella were subjected to phytochemical screening and anti-oxidant activity. Among the two samples seeds exhibits the maximum anti-oxidant activity.*

INTRODUCTION

In response to the increased popularity and greater demand for medicinal plants, a number of conservation groups are recommending that wild medicinal plants be brought into cultivation. Ethno pharmacological surveys conducted among herbal practitioners of traditional medicine in Palestine and the Middle East have revealed that a large number of indigenous plant species are being used as a source of herbal therapies. Medicinal plants are of noble worthiness to mankind. They are nature's offering for human beings to regulate a sickness free healthful life. They perform a necessary role in preserving our health (Bhagwati, 2003). Plants have been the basis of traditional medicines throughout the world for years and continue to provide new remedies to mankind (Kaewseejan *et al.*, 2012). Hence, in the present study phytochemical constituents and antioxidant activity of medicinal plants of C*aesalpinia bonducella* were studied.

MATERIALS AND METHODS

Collection of plant samples

Fresh plants Materials of *C. bonducella* were collected from different agro-climatic regions of Palani, Dindugul district, Tamilnadu from natural strands. Fresh samples were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Crude extract preparation

Fifty, grams of air- dried powder was taken in 200 ml of petroleum ether/ Ethyl acetate/ Ethanol/water in a conical flask, plugged with cotton wool and they were shaken at room temperature for 2 days. After 2 days of incubation, the supernatant was collected and the solvent was evaporated to make the final volume one fourth of the original volume and stored at 4 ^{0}C in airtight bottles.

Screening of phytoconstituents

Preliminary phytochemical analysis of various extracts

Qualitative phytochemical test were carried out in aqueous and alcoholic extracts of *C. bonducella* using standard procedure to identify the constituents by Trease and Evans (1989) and Harborne (1973) Methods.

Antioxidant activity

The antioxidant activity of crude extracts was determined by the following in-vitro methods.

Phospo- molybdenum assay

The total antioxidant capacity of the extracts were evaluated by the Phospho-molybdenum method described by (Ali *et al.,* 2008).The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate /Mo complex at acidic pH.0.3 ml each extract (6%) in triplicates were combined with 3 ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM Ammonium molybdate). The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. Methanol (0.3ml) in the place of extract was used as the blank. The antioxidant activity is expressed as the number of gram equivalent of Ascorbic acid. The calibration curve was prepared by mixing ascorbic (1000, 500, 250, 125, 62.5 and 31.25µg/ml) with methanol.

Nitric oxide free radical scavenging assay

Nitric oxide free radical scavenging assay was carried out as follows. Sodium nitropurusside (10Mm) in phosphate buffer (pH 7.7) was incubated with 900µg/ ml of each extract (6%) dissolved in their respective solvents in triplicate, and the tubes were incubated at 25 for 120 min. After incubation, 0.5 ml of the rection mixture was diluted with 0.5 ml of griess reagent (2% ortho phosphoric acid, 1% sulphnilamide, 0.1% napthylethylenediamine). Ascorbic acid was used as the standard. The absorbance of the pink chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with N-napthylethylendiamine was measured at 546 nm against the corresponding blank solution. Difference in obsorbance between the control and sample which expressed as percentage free radical scavenging of the nitric oxide by the extract (Hou *et al.,* 1996).

Ferric Reducing Antioxidant Power assay (FRAP)

3.6 mL of FRAP solution (0.3 M of Acetate buffer – pH 3.6; 10 Mm of TPTZ in 40 mM of HCL and 10 mM of Fecl3

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6H2 O) is added to distilled water (0.4 ml) and incubated at 37°C for 5 min. Then this solution mixed with certain concentration of the plant extract (80 ml) which was measured at 593 nm. For construction of the calibration curve, five concentrations of feSO4, 6H2O (0.1, 0.4, 0.8, 1, 1.12, 1.5 mM) were used and the absorbance values were measured as for sample solutions (Benzie et al., 1996).

RESULTS AND DISCUSSION

It is well recognized that free radicals are critically involved in various pathological conditions such as cancer, cardiovascular disorders, arthritis, and inflammation and liver diseases. Under normal physiological conditions, low concentrations of lipid peroxidation products are found in tissues and cells. In the presence of oxidative stress, more lipid peroxidation products are formed due to cell damage. Cellular antioxidant enzymes such as superoxides dismutase, glutathione peroxidase and catalase normally challenge oxidative stress.

Collection of plant samples

In the present study, the leaves and seeds were collected from the natural strands of Palani, Dindugul district, Tamilnadu, South India. After collection, phytochemical screening and antioxidant (Phospho-molybdenum assay, Nitric oxide free radical scavenging assay and FRAP assay) properties were quantified and the results were gives in table 1-4.

Phytochemical screening

Ethnol and Aqueous extracts of the selected samples were subjected to qualitative organic analysis (Table 1). The phytoconstituents of alkaloids, flavonoids, steroids and saponins are positively answered in both extracts of leaves. Tannins and terpenoids are present only in aqueous extract. While alkaloids, tannins, steroids, terpenoids and saponins are positively answered in both the extracts of seeds. Flavonoids are totally absent in both the extracts. Similar phytochemicals studied *Meliah azadiarch* and *Calotropis gigantea* (Argal and Pathak, 2006). The medicinal value of the presently studied samples are mainly attributed by the presence of phytochemical substances that exhibit definite medicinal effect in the human body.

Antioxidant activity

Phospho-molybdenum assay

The results of total antioxidant activity (TAC) by phosphomolybdenum assay is shown in the Table-3. It is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate /Mo (V) complex at acidic pH. TAC of the phospho- molybdenum model evaluates both water-soluble and fat-soluble antioxidant capacity (total antioxidant capacity).The results indicate a concentration dependent manner. It means that ethanol and aqueous extract of *C. bonducella* will have as much quantity of antioxidant compounds as equivalents of ascorbic acid of effectively reduce the oxidant in the reaction matrix. The extracts of a all the tested plant specimens possess total antioxidant activity ,but varying degrees, ranging from 0.011 to 0.063 mg/ml. which is an agreement with that of the previous reports on *Stachys iavandulifolia*, *Ocimum basilicus* (Abdul *et al.,* 2012).

Nitric oxide free radical scavenging assay

The nitric oxide free radical scavenging assay of plant sample was given in Table 4 which was found to be ranged from 13.81 to 26.88%. Among the two different parts, the ethanol extract of seeds (26.88%) exhibited the highest rate of free radical scavenging activity. Similarly the petroleum ether extracts of *Rivea hypocrateriformis* and *Hygrophila auriculata* showed anti-oxidant activity

FRAP assay

The total antioxidant activity of the studied plant sample is shown in the table 5. The extracts showed considerable antioxidant activity ranging from 0.017 to 0.538. The maximum total antioxidant activity was observed in aqueous extract of seeds.

Table - 1. Preliminary phytochemical screening of Ethanol and Aqueous extracts of *C. bonducella* Leaves and Seeds

S.NO	Phytoconstituents	C. bonducella			
		Leaves		Seeds	
		Ethanol	Aqueous	Ethanol	Aqueous
1	Alkaloids	+	+	+	+
2	Flavonoids	+	+	-	-
3	Tannins	_	+	+	+
4	Steroids	+	+	+	+
5	Terpenoids	_	+	+	+
6	Saponins	+	+	+	+

(+) Indicates Positive (—) Indicates Negative

Table- 2. Phospho- Molybdenum Assay of Ethanol and Aqueous extracts of *C. bonducella* leaves and seeds*

Plant Parts	Total antioxidant activity (mg Ascorbic acid equivalent / ml sample)	
	Plant extract	
	Ethanol	Aqueous
Leaf	0.013 ± 0.007	0.035 ± 0.020
Seed	0.011 ± 0.006	0.063 ± 0.036

* Data are mean of three replicates, ± Standard Error

Table –3. Nitric oxide free radical scavenging assay in Ethanol and Aqueous extracts of *C.bonducella* leaves and seeds*

Plant Parts	Radical scavenging activity (%)	
	Plant extract	
	Ethanol	Aqueous
Leaf	21.71 ± 0.04	13.81 ± 3.78
Seed	26.88 ± 0.094	19.01 ± 0.39

* Data are mean of three replicates, ± Standard Error

Table – 4. FRAP assay Ethanol and Aqueous extracts of *C.bonducella* Leaves and Seeds*

Plant Parts	Total antioxidant activity (mg $FeSO_4$ equivalent / g sample)	
	Plant extract	
	Ethanol	Aqueous
Leaf	0.238 ± 0.137	0.017 ± 0.010
Seed	0.126± 0.07	0.538 ± 0.310

* Data mean of three replicates, ± Standard Error

Conclusion

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drug have been isolated from natural sources, many based on their use in traditional medicine. In the present study clearly indicated that ethanol extract showed better nitric oxide free radical scavenging activity in *C.bonducella* seeds at concentration of 1000 mg/ml of extract. Hence, these potential medicinal plants may be recommended for the

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isolation and structural elucidation of important active principles, after conducting the suitable advanced studies, for the effective utilization of this plant.

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